# EFFECTS OF DILUENTS, AGE OF SEMEN AND INSEMINATION DOSES ON VIABILITY AND FERTILITY OF TURKEY SEMEN PRESERVED AT AMBIENT TEMPERATURE

**BY**

**ABUTU JOHN AUGUSTINE**
**PG/MSC/07/42510**

**DEPARTMENT OF ANIMAL SCIENCE**
**UNIVERSITY OF NIGERIA**
**NSUKKA**

**DECEMBER, 2011**

## CERTIFICATION

This is to certify that the work contained in this project was done by Abutu John Augustine Reg.No.PG/MSC/07/42510.The work is original and has not been submitted in this or any other university.

| | |
|---|---|
| **PROF.S.O.C UGWU** | **DR.A.E.ONYIMONYI** |
| **Project Supervisor** | **Head of Department** |

**EXTERNAL EXAMINER**

**December, 2011**

## DEDICATION

This work is completely dedicated to my dear wife Mrs. Agnes Eleojo Abutu and my son Mr. Joel Austin Abutu for their perseverance, moral and prayerful support towards my academic pursuit.

## ACKNOWLEDGEMENTS

I wish to acknowledge the tremendous contributions of Prof. S.O.C. Ugwu, my project supervisor, for his advice and continuous encouragement, which enabled me to produce a work of this quality.

My profound gratitude also goes to my Head of Department, Dr.A.E.Onyimonyi, Prof.C.C. Nwosu, Prof.A.G. Ezekwe, Dr. A.Ani, Dr.N.S Machebe and Rt.Com.Richard Gbande for their concern over my progress. Their various contributions really enhanced my ability to produce this work.

I indeed appreciate the great cooperation of Ogbadibo Local Government of Benue State for granting me permission and financial assistance to embark on this academic programme.

Finally I thank the Almighty God who provided all the human and material resources that made this work possible.

# TABLE OF CONTENTS

# ABSTRACT

An investigation was conducted with a total of one hundred and fifty (150) semen samples collected randomly from six (6) toms and one thousand three hundred and fifty eggs (1350) from forty-five (45) hens to evaluate the effect of diluents, age of semen and insemination doses on viability and fertility of turkey semen preserved at ambient temperature. Results of the work revealed that the semen qualities of those toms used for the work were not statistically different ($P>0.05$). The rate of semen survival per hour shows that in diluent (DIII) containing coconut milk a highly significant difference ($P<0.01$) in rates of survival of sperm during the 8 hour period was observed. Other diluents preserved sperm for a reasonable period of time, diluent (D0) lasted for 4 hrs, DI lasted for 5 hrs whereas DII also sustained survival of sperm for above 6 hrs before the sharp decline. The level of fertility recorded in all the diluents using different dosages showed that diluent D0 containing 0.2ml recorded the highest yield. The overall result in this case showed a highly significant difference ($P<0.01$). In the aspect of hatchability, diluent DI produced the highest number of chicks valued 63.48+8.27% .Significantly higher ($P<0.01$) hatch of fertile eggs and eggs set was obtained from hens inseminated with semen diluted in DIII than other diluents. From the work, it is evident that metabolizable substrates containing coconut milk and other diluents used proved efficient. The results show that careful improvement and utilization of those local and affordable diluents would enhance turkey semen preservation and artificial insemination practice in our local environment.

# LIST OF TABLES

# CHAPTER ONE

# INTRODUCTION

## 1.1 Background of the study

Turkey, a large poultry bird, is fast gaining popularity among peasant farmers in the country to the extent that they are produced throughout the year for commercial purpose. All over the world Turkey is produced by breeders due to its body size, comformation and edible meat yield. Reproductive traits like its oviparous nature and low to medium fertility have not prevented a reasonable rate of improvement under natural condition even in developing countries (Esminger, 1977).Following the quest for massive production of animals like pigs, rabbit and poultry with short reproduction cycle as a major remedy to acute animal protein shortage in Nigeria, emphasis is placed on poultry and its products because they are acceptable to almost all peoples, religions and communities. Among all the species of poultry in Nigeria, turkeys appear to be the less distributed with a population of (0.2 million) (FDLPCS, 1992).

However, it is highly cherished due to its large body size and high meat quality. In Nigeria, about 90% of turkeys are produced for Christmas market mainly for these reasons (Smith, 1990; Veg.Soc.2007).

Although this may be the case, the scanty distribution of turkey in the peri-urban and rural areas of the country calls for serious concern. A notable reason for low distribution of turkey could be its reduced fertility compared to chicken, which factor places serious constraints to its rapid development

especially under peasant farming conditions. The natural characteristic of male turkey (large body size) often results to reduced libido and low fertility which discrepancies cause toms not to mate frequently thereby resulting to low reproductive efficiency in turkeys under natural mating conditions.

The development of artificial insemination technology over the past decades has resulted in some significant advances in Turkey breeding. The objective of turkey artificial insemination programme is however not to produce fertile eggs but to produce viable poults (Murray, 1993). The US turkey industry relies on artificial insemination for the production of 300 million turkey annually (William, 2003). In developed world where artificial insemination is exclusively used to achieve acceptable level of fertility, donor toms as a rule are subjected to semen quality evaluation for fertility prediction (Donoghue, 1998). This idea coupled with the improvements on the technique of avian semen storage at ambient temperature, has led to increased multiplication of turkey in developed parts of the world. The situation is rather different in developing countries where these techniques are not adopted and producers have to rely on natural mating and egg hatching techniques to produce poults. Nigeria is one of such developing countries where the use of Artificial Insemination (AI) is still at the experimental stage such that turkey poults are imported for rearing and natural methods are applied on existing local stock to produce poults for turkey meat production.

## 1.2 Statement of the problem

It is well known that male breeders (stags) are usually bred for large body size and become too broad breasted and heavy to mate naturally with the comparatively smaller hen. The accompanying results in the inability of males to complete mating sessions successfully, causing low fertility under natural mating condition. Apart from this, ejaculated turkey spermatozoa is reported to perish after several minutes outside its own seminal fluid (Free potent soline.com 2001) and rapidly lose viability and fertilizing capacity when stored either undiluted or diluted at physiological temperatures (Leighton *et. al.,* 1969; Lake and Ravie, 1982). AX *et al.* (2000) also reported that the survival of turkey ejaculated sperm in seminal plasma alone is limited to a few hours. As such its fertilizing ability is impaired or hampered and this often results to low production of poults.

In turkey breeding there is need to prolong the viability and duration of the fertilizing capacity of turkey sperm for genetic improvement and economic advantage. There is also the need to have repeatable techniques necessary to maintain the fertilizing potential of ejaculates from 6 to 24 hours under field condition. It is necessary to develop effective techniques for semen collection, dilution and insemination in the field to solve the problem of infertile mating under natural conditions for the benefit of small and large scale turkey producers in the country.

The use of Artificial Insemination technique in commercial poultry production is relatively recent compared to its use in cattle production. It has been applied mainly in turkey breeding and used to a lesser extent in chicken (Sexton, 1979).

The gap created by minimum use of this technique in the poultry industry in the tropical environment must be breached for maximum productivity especially in the turkey species.

The present study is therefore designed to test the survivability and fertility of turkey sperm stored under ambient temperature in different dilution media containing different types of metabolizable substrates including tropical coconut milk and citrus juice without antibiotics.

**Objective of the study**

This research project is designed to study the survivability of turkey sperm preserved in different diluents and to determine the fertility of ejaculates using different insemination doses.

The specific objectives of the study are:

1. To determine the effect of different diluents on survivability of ejaculated turkey sperm.
2. To determine the effect of insemination doses on fertility and hatchability of turkey eggs.

## 1.3 Justification of the study

Low fertility in turkeys, due to unsuccessful mating caused by large body size of the tom and reduced libido is a serious and costly problem in the production of hatching turkey eggs (Merck pub., 2008).

Burke (1984) observed that modern toms lack the coordination and dexterity to complete mating to assure high fertility. In most cases partial completion of mating act even without transfer of semen to the female results in variable periods of sexual refractoriness during which time hens normally will not remate.

Apart from this, lack of efficient storage or dilution media is a contributory factor since low number of spermatozoa from superior sires can be propagated if ejaculates are properly extended in good diluents. Similarly, lack of adequate knowledge on usage of natural substrates like orange juice, coconut milk and inclusion of antibiotics during semen preservation has added depletion value to semen storage as well as relegated genetic potentials of toms. These inadequacies have adversely affected poult production and economic viability of turkey industry.

In this country, different natural metabolizable substrates like orange juice, coconut milk, tomato juice are available at low cost to assist turkey farmers in preserving semen under ambient temperature. To save our turkey industry from collapse, efficient semen dilution and preservation technique must be employed to facilitate high fertility of turkey semen in the field. In the same

vein, improvements leading to long-term storage of turkey semen are important especially now that the commercial production of domestic turkey rely almost entirely on artificial insemination.

The present study is aimed at facilitating effective large scale production of turkeys by artificial insemination to improve the low per capita protein intake in the country boost the economic viability of turkey farming.

# CHAPTER TWO

# LITERATURE REVIEW

## 2.1 Origin and domestication of turkeys

Native to North America, turkeys were first domesticated more than 2000 years ago in Mexico, South America. In 1910, the US turkey industry was comprised of 8,700,000 farmers raising turkeys at an average of four birds per farm. Turkeys are typically free- ranging and exist in every environment, which permits them to display normal behavior patterns. In the 2000 millions of turkeys were slaughtered in the United States for meat and more than half were raised under contract for just three companies. Today, domesticated turkeys have been selected to grow efficiently and reach much height and weights than their wild counterparts in exceedingly shorter periods of time (An HSUS Report, 2000-2007).

### 2.1.1 Origin of turkeys

Turkeys are native to America. They were found in great numbers by the pioneer settlers, and a limited number of wild turkeys still exist in certain remote areas. Turkeys were also plentiful in Mexico (Esminger, 1977). It is reported that turkeys were taken from this continent to Spain in 1498 and to England soon thereafter. Later, some of the European stocks were brought back to America where along with the native wild turkey, they were used in developing the present varieties.

### 2.1.2 Genetic make up of turkey

Turkey can be classified by their breeds or plumage colur (Thear, 2007). Several plumage colors of turkey exists which include black, brown, red and white (Sccherger, 1964) with black, bronze and white predominantly existing in the tropical environment.

### 2.1.3 Breeds of turkey

Initially, Turkeys are all of the traditional narrow breasted phenotype with prominent long legs. A survey of males and females revealed that males weighed 11-13kg and hens 5.9 - 7.2kg. Examples of common color phases are black and bronze but other varieties such as red, buff grey and variegated birds are also present. Some breeds of turkey currently being reared include:

**Broad Breasted Bronze**

According to CPDO (2009), the basic plumage color of this turkey is black not bronze. The females have black breast feather with white tips, which help in sex determination as early as 12weaks of age.

**Bourbon Red Turkey**

This was developed in Bourbon country, Kentucky. It is probably the most attractive variety. It is reddish-brown in color and has white primary, secondary and main tail feathers. This was developed in late 1900s from Buff, Bronze, and white Holland. It was admitted to standard in 1909 (The perfect-Turkey.com., 2007).

**Beltseville small white**

This variety was introduced in 1941.It close resembles the Broad Breasted white in color and shape, it is noted for its small size and thick, meaty breed. Egg production, fertility and hatchability tend to be higher and broodiness tends to be lower than heavy variety (CPDO, 2009). In today's industry this type of turkey is no longer utilized (Charlotte *et. al*,.2011).

**Slate:**

This is also called Blue Slate. It is named for it genetic mutation and was admitted to standard in 1874 (The Perfect-Turkey.com.2007).

**Black:**

Bred in Europe from North America Wild Turkey stock and brought to America. It was admitted to standard in 1874. This turkey breed is also called Spanish Black and Norfolk Black (The-Perfect-Turkey.com.2007).

**Narragansett Turkey**

The Narragansett has plumage with black, grey, tan, and white feathers. It resembles the Bronze Turkey but has feathers of grey or dull black replacing the bronze turkey's distinctive coppery coloring. It sometimes has bars of white feathers on its wings due to a genetic mutation not found outside the United States. It has a black beard, a horn-color from red to bluish white. The breed is prized for its excellent temperament combining a calm disposition with good maternal abilities. They mature early, are good egg producers, have excellent quality meat and when kept at liberty do wander far from home. Toms weigh

10-12.7 kg and hens weigh 5.4-7.3 kg. They can run quickly, fly well, and prefer to spend their nights roosting in trees (Wikipedia, 2009).

**Local turkey**

According to Ajala *et .al.* (2007) local turkeys are foragers and can be kept or reared in different parts of the country especially in the savanna and derived savannah belts. They are small in size and exhibit slow growth rate.

## 2.2 Commercial value of turkey

Turkey are used as a major source of protein (meat and eggs), and also the feathers are exclusively used for decorative purposes as did many other meso- American cultures (Wikipedia-Domestic Turkey, 2009). Although turkey is reared mainly for festivals such as Christmas its meat is now eaten year round and forms a regular part of many diets. They may be sliced or ground, as well as prepared" whole" in a manner similar to chicken with head, teat and feathers removed. Sliced turkey is used as a sandwich meat or served as cold cuts while ground turkey is sold just as ground beef. Turkey litter (droppings mixed with bedding material, usually wood clips) is being used as a fuel source in electric power plants. One of such plants in western minnetola provides 55 mega Watts of power using 500,000 tons of litter per year (Domestic Turkey, 2009).

## 2.3 Nutritional value of turkey

Turkey serves as food as well as breast meat because it has high nutritional value, low fat content and affordable price. According to German

(2001) turkey meat is delicious and lends itself well to a variety of menus. Turkey meat can be seasoned and formulated into numerous products. The dressing yields in turkey surpass chickens by a considerable margin. Details of nutritional value of turkey are shown in the Table 1.

**Table 1: Nutritional value of turkey**

| **Nutritional Value per 100g(3.5oZ)** | |
|---|---|
| Energy | 436kj(104 kcal) |
| Carbohydrate | 4.21g |
| Sugars | 3.51g |
| Dietary Fiber | 0.5g |
| Fat | 1.66g |
| Protein | 17.07g |
| Thiamine (Vit.B1) | 0.130mg (10%) |
| Riboflavin (Vit.B2) | 0.320mg (21%) |
| Niacin (Vit.B3) | 0.110mg (1%) |
| Pantothenic acid (Vit.B5) | 0.166mg (3%) |
| Vitamin B6 | 0.128mg (10%) |
| Folate (Vit.B9) | 4Ug (1%) |
| Vitamin C | 5.7mg (10%) |
| Calcium | 8mg (1%) |
| Iron | 1.44mg (12mg) |
| Magnesium | 21mg (6%) |
| Phosphorus | 162mg (23%) |
| Potassium | 302mg (6%) |
| Zinc | 1.33mg (13%) |

*Percentages are relative to US recommendations for adults.*
*Source: USDA Nutrient Database (2009)*

## 2.4 Reproductive characteristics of turkey

In males although semen may be produced as early as 16weeks in white leghorn and 28weeks in turkeys, there is rarely sufficient semen of good fertilizing capacity until cockerels are 28 weeks old and stags 32-36 weeks old (Marire, 2011). According to German (2011) turkeys reach sexual maturity at an older age than chickens. For this reason they are generally not light stimulated until they are between 29 and 32 weeks of age. Hens are managed to produce first eggs at about 32 to 36 weeks of age and the production cycle lasts for 6 months. Thear (2005) reported that they attain laying stage at around 28weeks onward depending on the breeds, with breeding season lasting from 16 to 20 weeks. Lighter breeds can lay up to 100 eggs in a season and come into lay earlier, whereas the heavier types may lay as far as 50 eggs. Turkeys normally lay between April and June although there may be some eggs laid in March and July. The incubation period of turkey egg is 28days. The poults are normally removed from the hatchers on the $29^{th}$ day.

**Table 2: Comparative reproductive performance of some birds**

| Species | Incubation period (days) | Age at sexual maturity (mnths) | Egg weight (gm) | No. eggs in first laying year (%) | Fertility (%) | Hatchability of fertile eggs (%) |
|---|---|---|---|---|---|---|
| **Chicken** | | | | | | |
| Layer | 21 | 5-6 | 58 | 300 | 97 | 90 |
| Broiler | 21 | 6 | 65 | 180 | 92 | 90 |
| Turkey | 28 | 7-8 | 85 | 90 | 83 | 84 |
| **Duck** | | | | | | |
| Layer | 27-28 | 6-7 | 60 | 300 | 95 | 75-80 |
| Meat type | 28 | 6-7 | 65 | | | |
| **Goose** | | | | | | |
| Small type | 30 | 9-10 | 135 | 30-70 | 70 | 70 |
| Large type | 33 | 10-12 | 215 | | | |
| **Pheasant** | 24-26 | 10-12 | 30 | 50-75 | 95 | 85 |
| **Guinea fowl** | 27-28 | 10-12 | 40 | 80-200 | 90 | 95 |
| **Quail** | 15-16 | 1.5-2 | 10 | 300 | 90 | 75-85 |

Adopted from: Reproduction in farm Animals by Hafez E.S.E 5th edition (1985)

## 2.5 Artificial insemination Technique in Avian Species

The Macquarine Encyclopedia defined Artificial Insemination as a method of inducing pregnancy by artificial introduction of viable sperm into the canal of the cervix. Available reports revealed that the earliest Artificial Insemination was in 1780 when Spallanzani, an Itallian Physiologist obtained pups by this method. Other scattered reports appeared in the 19$^{th}$ century but it was not until about 1900 that extensive studies with farm animals began in the USSR (Foote and Trimberger, 1968). Since then Artificial Insemination has been used in animals like cattle, goat, pig and others in different parts of the world like Russia, Britain and Denmark.

In the avian, it is a method of inducing fertility by assisting with the introduction of viable sperm into the vent of an avian species for the purpose of selective breeding (Budgerigar soc.Inc., 1999). Essentially, 100% of the nearly 300million turkeys produced annually in the United States of America for consumption are the result of artificial insemination (Tara weaver, 1998). Insemination is accomplished with the aid of an assistant who everts the cloaca to reveal the oviduct. .

Donoghue (1999) indicated that artificial insemination of turkey is considerably more efficient than natural mating given the sheer number of hens that are needed to be inseminated. Another important benefit of turkey Artificial Insemination is that fewer toms are needed to keep hens producing fertile eggs.

### 2.5.1 Artificial insemination in turkey

Artificial insemination in turkey began in 1930 when Beltsville based researchers William Burrow and Joseph Quinn reported ground breaking methods for semen collection and Artificial Insemination for poultry. In the 1970s, Thomas J. Sexton developed the Beltsville poultry semen extender-a solution that dilutes and preserves the sperm outside the bird's body. Since then Artificial Insemination in turkey has continued to flourish and this has aided turkey farms since large number of poults can be gotten through Artificial Insemination. Its use in turkey breeding is wide spread due to the development of heavy, broad breasted bird with a conformation which makes natural mating difficult. As a result, it has become the only method for economic poult production and some 95% of this success results from an Artificial Insemination breeding programme (Marire, 2011) .Important too is the decrease in ratio of toms from 1 to 10 with natural mating to 1 to 30 with Artificial Insemination (Tara Weaver, 1998).

Most researchers now focus on evaluating semen quality/ function and improving ability of each tom's sperm to actualize important breeding programme. The sperm motility test enables us to objectively measure how well sperm from each male can swim in a solution at body temperature, possibly mimicking the environment in the hen's reproductive tract .It is possible to identify good quality sperm since males with the trait for fast-moving sperm carry that trait through time .It is also important to understand how their sperm

differ by understanding which physiological characteristics influence sperm fertility .

## 2.6 Semen Quality

The semen quality as determined in the laboratory takes into account parameters which are used in predicting semen fertilizing ability. These parameters include Semen volume, sperm concentration and the percentage of live sperm and proportion of morphologically normal spermatozoa (Liu *et al*., 2009). Traditionally poultry semen evaluation methods used in the industry for selection of semen donors either have not been feasible to perform on individuals or have not been predictive of fertility (Donoghue, 1998).

### 2.6.1 Semen volume

In semen evaluation, the importance of semen volume cannot be over emphasized. This is necessary to calculate the appropriate rate of dilution to obtain about 100million cells per insemination. Variations in ejaculate volume of birds usually exist between species, breeds and individuals within a flock. Other factors include, age, frequency of ejaculation, season of the year, extent of massage or handling before and during massage (Perry, 1962; Rose, 1997 and Etches, 1998). The volume of domestic cocks ranges from 0.24 to 0.80ml, thus indicating that domestic birds produce small amount of semen compared with other farm animals. The volume of turkey semen ranges between 0.2 to

0.5ml.Small semen volume is not harmful but if accompanied by a low sperm concentration, the number of sperm available is limited (Hafez, 1987).

### 2.6.2 Sperm motility

Moss *et al.* (1970) established that, sperm motility measures the percentage of spermatozoa that are progressively moving. That is to say, sperm cells that move forward in a straight line are counted and not those undergoing circularly, backward or vibratory movement. This assertion was made based on the microscopic method of semen study. Sperm motility enables us to objectively measure how well sperm from each male can swim in a solution at body temperature, possibly, mimicking the environment in hen's reproductive tract (Tara, 1998). Donogue (1995) reported that it is possible that sperm motility influences the number of sperm in storage tubules in the hen and subsequently fertilize the egg. Motility could be estimated by hanging drop method at 400x magnification (Bakst and Cecil, 1997).The commonest and easiest method of estimating sperm motility is the subjective method of rating semen based on the swirling motion observed when fresh semen is examined under a light microscope. The 0 to5 scale proposed by Herma and Swanson (1941) (0 for no motility and 5 for highest motility) has been used by Tarada (1983).Other methods in determining the motility of sperm include the microscopic, mathematical and photoelectric methods.

According to Wheeler and Andrews (1943) the following scale for motility rating/evaluation was presented as shown in Table 3 below:

**Table 3: Motility rating scale**

| **Motile cell** | **Descriptive value** | **Numerical score** |
|---|---|---|
| 0-1 No-motility | - | - |
| 1-20 Percentage motility | Very poor | 1 |
| 20-40 Percentage motility | Poor | 2 |
| 40-60 Percentage motility | Fair | 3 |
| 60-80 Percentage motility | Good | 4 |
| 80-100 Percentage motility | Very good | 5 |

Motility rating scale (Wheeler and Andrews 1943)

The Table above clearly shows that the percentage motility values above 60 percentage is indicative of semen with more active sperm cells which may possess good fertilizing potential. Etches *et al.* (1998) also ranked motility on a subjective scale of 1-5 or 1-10, where the bottom of the scale represents poor motility, the middle of the scale representing average motility and the top of the scale representing excellent motility**.**

### 2.6.3 Sperm concentration

Poultry semen is viscous and highly concentrated, containing sperm concentration of 6 billion to 12 billion sperm per ejaculate for rooster and tom respectively (Donoghue and Wishart, 2000). Sperm concentration is an

important measure of semen quality and provides information on the extent of dilution necessary to obtain required sperm numbers per insemination dose. Donoghue *et al*. (1996) acknowledged the importance of determining sperm concentration of ejaculates. Brillard and McDaniel (1985) outlined the following methods for determining the concentration of sperm cells of an ejaculate.

(1) Haemocytometer count (2) Use of spectrophotometer (3) Use of counter (4) Use of spermatocrits

Another method that is presently in use is the microprocessor controlled semen analyzer(Densimeter,Model 534-B-Model,Animal Reprod.systems) initially used for evaluating stallion semen but now modified to evaluate poultry semen (Donoghue *et al*., 1996). This method provides accurate and precise data on sperm concentration beneficial to artificial insemination.

Etches (1998) noted that the concentration of sperm in semen of domestic birds is higher than that of cattle, pigs sheep and goats. Turkeys produce less semen volume than chickens but the concentration of spermatozoa is much greater. The mean volume of turkey semen is about is 0.2ml per collection but the concentration varies from 6.2 to 7m per cubic millimeter depending in breed (Mc Cartey and Brown, 1959). Merck & Co (2004) reported that the concentration of 0.025ml contains 2 billion sperm). It maintained further that the semen of turkey contain the volume of 0.35-0.5ml with a spermatozoa concentration of 6->8 billion/ml.

### 2.6.4 Semen pH

Rose (1997) identified fresh semen to be alkaline with an average pH ranging from 7.0 to 7.6. During storage semen pH depends on the various proportions of several secretions in the semen especially the transparent fluid. For instance, semen containing bacteria and many dead spermatozoa as contaminants may evolve ammonia which decreases the pH of the samples (Salisbury and Mercies, 1945). During long storage of spermatozoa, semen pH decrease thus leading to a reduction of spermatozoa motility. This could be as a result of an increase metabolic rate leading to production of lactic acid anaerobically. Semen pH has been determined using pH meter and bromothymol blue paper.

## 2.7 Spermatozoa morphology

Avian spermatozoa according to Etches (1998) is about 0.5um at its widest point and approximately 100ul in length with a volume of about $10\mu m^3$.The spermatozoa of avian species are longer than mammalian, but the head is narrower. The spermatozoa are torpedo shaped rather than paddle shaped. Chicken and turkey spermatozoa are filiform in shape (Bask, 1980 and Lake, 1981). They are rarely indistinguishable by light and scanning electron microscopy. They have simple acrosome with the mid piece being a cylindrical of distal centroile surrounded by a cover of mitochondria.

The chemical and physical properties of avian semen differ from those of mammals probably because of the absence of seminal vesicles and prostate glands. The seminal plasma has little fructose, citrate, inositol, phosphatidylcholine, ergothionine, and glyceryl phosphorylcholine. Potassium and glutamate levels are high, while chloride levels are low (Carman *et.al.*, 2004).

## 2.8 Sperm Abnormality

Several deviations from the normal sperm morphology are regarded as abnormalities. Abnormalities according to Etches (1970) are classified into three categories based on their source and nature of incidence.

**Primary abnormalities:** These are due to defects in the seminiferous tubules and caput epididymis. Examples include structural deviation of the head, the middle piece and immature sperm cells.

**Secondary abnormalities:** These occur due to degenerative changes in the sperm probably due to prolonged stay in the tail epididymis. Degeneration of sheath lining the head and mid-piece, broken necks, detached heads, and burst heads are examples of secondary sperm abnormalities.

**Tertiary form:** occur due to poor or improper laboratory handling of semen. Examples of tertiary forms of abnormalities include broken tail, coiled tails, broken necks etc formed as a result of faulty dilution, cold shock and crushing or spermatozoa.

## 2.9 Pooling of semen

The practice of pooling semen from several males in the field is an acceptable procedure since semen qualitative characteristics are not seriously affected (Shakle *et al.*, 1980). Pooling of semen helps to eliminate the effect of individual variability of gamete donor (Taner *et al.*, 2010). In artificial insemination pooling of rooster semen was encouraged because roosters are evaluated individually for reproductive potential at the time of collection beyond assessment of ejaculate color and volume (Hoolsberger *et al.*, 1998). In a work reported by scientists, hens inseminated with semen from multiple toms to prove DNA finger printing to determine the paternity of offspring showed a fruitful result. The result revealed that when semen from 7 to 10 toms was pooled, only 1 or 2 males produced a majority of the offspring (Tara weaver, 1998).

## 2.10 Semen storage

Semen storage refers to the technique of storing or preserving semen for use during Artificial insemination. According to Garmen *et al.* (2004) semen could be frozen and thawed. Graham *et al.* (1982) used some extenders for turkey semen with a frozen-thawed recovery of greater than or equal to 50% motile spermatozoa and a vigorous swirl but detrimental effects occurred after some hours. In a work on semen preservation, Sexton and Gieson (1989) observed excellent sperm survival and hatchability when diluted turkey semen

was stored for 6 hours in a cool liquid state Beltsville Turkey Semen Extender before Artificial Insemination. However liquid storage beyond 6 hours or freezing of semen has not preserved the viability of turkey semen at the level necessary for commercial use (Sexton, 1998; Thurston, 1995). Similarly Akcay *et al.* (1997) adduced that turkey semen could be stored in a refrigerator for 24hours or 48hours at $4^0$C without appreciable loss in fertility. Gadea (2003) also reported that preserving semen at temperatures below $15^0$C would help reduce sperm metabolic activity and protect against the detrimental effects of microbial contamination.

Several factors must be taken into account when preserving spermatozoa. These include temperature, energy source, osmotic pressure, electrolyte balance, pH and buffering capacity, microbial control and appropriate dilution rate (Salisbury *et al.*, 1978).

Turkey semen may be kept at temperature range of 5-$15^0$C for few hours (6-18 hours) or frozen, but reduced fertility limits usage to special breeding programme (Merck & Co., 2009).Absolutely different results were obtained by Long and Kramar (2009) which indicated threefold increase of malondialdehyde MDA production in turkey semen stored in analogous condition in comparison to fresh semen.

### 2.10.1 Ambient temperature

Hot ambient temperatures above the thermoneutrality for domestic poultry, typify the summer season in the greater poultry production area

especially in tropical regions and these affect performance and overall adaptation to the climatic regions (IIorin *et al.*, 2009). Prolonged periods of elevated ambient temperature stress increase the time to reach market weight and increase mortality (Deaton *et al.*, 1978). According to Reece and Lott (1983) these conditions reduce feed intake and growth as well as feed efficiency in growing birds.

### 2.10.2 Storage of semen at ambient temperature

**Development of room temperature:** It is clear from the survival rates of sperm in the epididymis and the female reproductive tract that sperm cells remain viable at body temperature for a significant period of time (Marire 2011). Refrigerating semen at $5^0$C and freezing semen have greatly increased its in vitro storage life. The need for unrefrigerated semen has therefore diminished, but in some areas refrigeration is not widely available or is expensive. Hence storing spermatozoa in nutrient media at ambient temperature obviously makes it necessary to control bacteria. VanDemark and Sharma (1957) used IVT extender and reported little change in fertility when a limited number of cows were inseminated with semen stored up to seven days at ambient temperature.

Semen motility from both undiluted and diluted chicken semen is lowest when stored at $41^0$C which is near the body temperature of the hen. This is in contrast to semen stored at 25, 15 or $5^0$C (Dumpala *et al.*, 2006). Storage of semen at 0-$5^0$C for 4 hours reduced motility of fresh semen. Hafez (2001)

reported that fertilizing capacity (90% or more) can be maintained for up to 18 hours when stored at temperature lower than ambient temperature. Laffaldano *et al.* (2010) reported that different storage materials (bags, foils, glass and plastic tubes) may affect motility and fertility in cooled semen samples.

## 2.11 Diluents for Turkey semen

Diluent or extender refers to the aqueous solution used to increase the volume of the ejaculate to a level appropriate for making multiple inseminations (Picket and Berndtson, 1974; Graham 1978; Salisbury *et al.*, 1978; Foote, 1980). Undiluted semen dies or loses its fertilizing capacity in about 4-6 hours at room temperature. Its life may be slightly prolonged by slow cooling to $10^0$C (Gadea, 2003).

At a practical level and for current production purposes, diluents can be divided into two major groups. Those designed for short-term preservation (less than 1 to 3 days) and diluents for long-term semen preservation (over 4 days).

To perform its function, the extender must have the ability to supply nutrients needed for the metabolic maintenance of the sperm cell (glucose), afford protection against shock (BSA or egg yolk) , control the PH (bicarbonate, Tris, Hepes) and osmotic pressure (Nacl, Kcl) and inhibit microbial growth (Antibiotics).

### 2.11.1 Chemical composition/Biological value of Coconut

Coconut water is one of the world's most versatile natural products (Jean *et al.,* 2009). It is a refreshing beverage that is consumed world wide as it is nutritious and beneficial for health. Traditionally it is used as a growth supplement in plant tissue culture/micropropagation. Quality of coconut water can be justified by its unique chemical composition of sugar, vitamins, minerals, amino acids and phytohormones. It is a refreshing and nutritious beverage, used extensively as a growth-promoting component in tissue culture medium. Van overbeek *et al.* (1941) introduced coconut water as a new component of the nutrient medium. It has growth regulatory property e.g. Cytokinin-type activity.

**Coconut milk Extender:** Norman first used coconut (*Coco nucifera*) milk as part of extender for semen and he recorded that coconut milk had the advantage of sustaining livability of fresh semen (Norman, 1962). When used fresh, buck semen extended in coconut milk was reported to give appreciable sperm motility and fertility with acceptable conception rate (CR) post breeding. Like wise, bull semen extended in coconut milk citrate yielded appreciable conception rate in cattle when used fresh. Interestingly, Bakst (1981) and Reuden *et al*. (1981) noted that coconut juice act as metabolic substrates to maintain the functional integrity of spermatozoa during storage especially during in-vitro storage.

### 2.11.2 Biological and nutritional value of Orange juice

Orange juice is very nutritious and pleasant to take at anytime. It contains Vitamin C, Flavonoids, Anthocyanin. It does not contain fat but is nutritionally high in fructose and contains energy. Virtually 100% of orange juice provides a variety of vitamins and minerals. It is reported to be free from saturated fat, and cholesterol . (*www.orange juice facts.com/nutriti.htm, 2008)*.It is loaded with vitamin C. Besides , it is also a rich source of vitamin A and vitamin B. It also contains minerals like calcium, iron, sodium, potassium, magnesium, phosphorus, copper and sulfur. The nutritional value of orange juice is presented in Table 4.

**Table 4: Nutritional value of orange juice**

| **VITAMINS** | **MINERALS** |
|---|---|
| VitaminC-70mg | Potassium-273.11mg |
| VitaminB1(thiamine)-0.11mg | Calcium-52.4mg |
| vitaminA-269iu | Phosphorus-18mg |
| folate-39.7mg | Magnesium-13mg |
| Pantothenic Acid(vit.B5)-0.33mg | Selenium-0.65mg |

USDA National Nutrient Database for standard reference (2008)

Apart from these, trace minerals like copper, iron, sodium, sulfur, zinc and manganese are present. Orange juice also contains 15.4grams of carbohydrates, 12.2grams of sugar, 3.0 grams of dietary fiber, 1gram of protein

and 0.2grams of fat. Pectin, fiber found in the orange peel helps in lowering cholesterol levels.

Although there is paucity of information regarding the use of orange juice as semen extender, Bakst (1981) and Reuden *et al.* (1981) reported that it may act as metabolic substrate to maintain the functional integrity of spermatozoa during storage especially during in-vitro storage. Fruit juices such as orange and grape juice have been found suitable for bull semen extension (Bonadona *et al*., 1962).

### 2.11.3 Glucose

The choice of an extender is crucial given its profound effect on the economic viability of artificial insemination programme. Glucose extender was able to supply the nutrients needed for metabolic maintenance of the sperm cell (Gadea, 2003). Earlier findings indicated that simple glucose-base extender has been used in cooled storage of turkey semen from 24 to 48 hours at $4^0$C with minimal loss in fertility (Akcay *et al.*, 1997). Metabolism of glucose and acetate are lower in avian than mammalian spermatozoa and glycolitic enzyme activities of turkey sperms are 2-10 times lower than in the fowl (Scott *et al.*, 1962; Wishart, 1982).

## 2.12 Antibiotics

Over the years, antibiotics have been used as a feed additive in animal diet in order to increase feed efficiency, promote growth and for preventing and

treating diseases (Falcao-e-cunha *et al*, 2007). Therapeutic usage of antibiotics is typically a high dose-short term one, the substance is either injected, or administered via feed or water. Antibiotics are added to most semen diluents as a prophylactic measure against transmission of pathogenic bacteria and to reduce the level of non-pathogenic organisms that contaminate the semen (Alvai-shoutari *et al*., 2007).

In Artificial insemination drugs like benzylpenicillin, streptomycin and lincomycin were used as antibiotics to control bacterial such as mycoplasma and ricketsia. However it is evident that the efficiency of antibiotics may be reduced in the presence of some components of the diluents, notably egg yolk. Garner (1991) identified some problems with resistant organisms. Relatively, massive use of antibiotics in animal production has been implicated in the emergence of antibiotic-resistance pathogenic organisms, a growing public –health concern (Falcao-e-cunha *et al.,* 2007; Javaidel *et al*., 2008).

## 2.13 Dilution of semen

The dilution of semen in appropriate media not only provides an environment suitable for extended storage of the spermatozoa but also dilutes the sperm concentration to arrive at several insemination doses. Cupps (2001) reported that diluted semen at room temperature keeps its fertilizing capacity well for two hours and when cooled within 30 minutes to $5^0$C, it can be used for 12 hours. Semen is often diluted in artificial insemination practices to provide a suitable medium that will sustain and protect spermatozoa thus prolonging their

fertilizing capacity until they are used for insemination (Salisbury *et al*., 1978). Most American investigation have depended on dilution of the semen in order that the individual cells can be readily seen at x 200-400 magnification (Marire, 2011).

### 2.13.1 Semen dilution ratio

Obvious advantages of semen extension have generated interest in finding dilution rates for semen of avian species. Donogue (2000) advanced that the ability to obtain fertility results similar to natural mating was dependent on the rate of dilution. Sexton (1977) stated that high fertility could be obtained by inseminating chickens weekly with semen diluted at 1:4. Dilution levels of 1:5, 1:10 and 1:20 exhibited marked reduction in fertility compared with 1:2 and 1:3 and not even an increase in semen dosage for 0.1 to 0.2ml improved fertility in chickens at the 1:10 and 1:20 level because tom sperm concentration is higher than that of the rooster. Omprakash *et al.* (1992) reported that when semen was diluted 1:3 there was an increase in the percentage of abnormal spermatozoa due to dilution effect.

### 2.13.2 Effect of semen dilution

Dilution reduces the number of spermatozoa per ml of semen. Munro (1938) found a decline in sperm numbers with increasing dilution, but the decline is only appreciated when the dose of insemination contained less than the minimum number of spermatozoa required for optimum fertility (Brown,

1970; llinskii *et al*., 1975). Loss of fertility following excessive dilution of semen could result by inseminating chickens weekly with semen diluted 1:4. Sexton (1987) reported that fertility of stored turkey semen diluted 1:2 was lower than that of semen diluted 1:1 and 2:1.

## 2.14 Method of insemination

A Syringe is used to deposit semen at a depth of approximately 3cm. Similarly insemination apparatus of a 1ml glass and metal tuberculine syringe to which if fitted a 5-6cm plastic cannula is also used.

**Table 5: Showing method of insemination: fertility, egg production and mortality of hens after vagina or deep insemination.**

| Method of insemination | Semen Source | No of hens Initially | died | Hen days | Total | Fertilized |
|---|---|---|---|---|---|---|
| Intra-vagina | Turkey | 26 | 0 | 304 | 226 | 6 |
| Intra marginal | | 16 | 4 | 374 | 270 | 0 |
| Intra-uterine | Rooster | 7 | 0 | 42 | 15 | 15 |
| Intra-uterine | Turkey two suspension | 16 | 10 | 36 | 3 | 0 |
| Intra-uterine | Turkey six suspension | 51 | 6 | 268 | 114 | 24 |
| Intra-uterine | Turkey eleven suspension | 19 | | 564 | 166 | 36 |

*Adopted from: The fertilization rate of domestic hens after intramagnal and intra-uterine insemination with turkey spermatozoa (Orakempenichi-pinto 1970)*

### 2.14.1 Timing of insemination

Timing of fowl and turkey insemination is very important if high fertility levels are to be achieved. It is generally recognized that the operation should be

carried out when no hard shelled egg is likely to be present in the uterus or at least not within 3hours of an oviposition (Giesen *et al*., 1980; Christense and Johnson, 1975, 1977, 1978). The presence of an egg undergoing plumping in the uterus during insemination is compatible with achieving good fertility. However, the proper insertion of semen may be impeded when a hard-shelled egg is in the uterus and some spermatozoa may be washed out of the vagina if an egg is oviposited soon after insemination before they gained access to the utero-vagina storage glands.

Chickens are inseminated at weakly intervals, whereas the turkey requires insemination at 2 to 3 week intervals (Lake, 1962; Hafez, 1987). Marire (2011) pointed out that insemination of chickens twice weekly could lead to maximal fertility. But for good results in turkey insemination of hens at 1, 2 or 3wks interval is ideal. Hen are inseminated late in the afternoon between 2 to 3p.m to avoid the presence of a hard shelled egg in the uterus

### 2.14.2 Insemination dosage

The semen doses that can effect fertility vary according to species. There is an inverse relationship between the concentration of the sperm in the semen and the required dosage .Doses for insemination with undiluted semen are for chicken 0.1ml,turkey 0.05ml,for duck 0.3ml and goose 0.05ml.Avaible information revealed that different dosages had been used to effect fertility in poultry and other animals. For example, Lukaszewics (2001) reported the use of

0.2ml and obtained fertility result of 66.1% in Turkey,82% fertility result was reported in duck using 0.3ml by Bunaciu *et.al.* (1990) and a work by Aflonuv (2001) yielded a significant effect ($P<0.05$*).*

### 2.14.3 Handling of hen's prior to insemination

Rough treatment of hens must be avoided during capture before insemination and each female must be released gently after insemination otherwise semen may be regurgitated from the vagina. Macherson *et al.* (1977) reported a lowered fertility in hens which were thrown down on to litter after insemination. Meyer *et al*. (1980) suggested that females might be disturbed or distracted in egg laying process if handled too frequently, especially towards the end of breeding period.

The authors (Meyer *et al., 1980*) found that handling hens weekly for artificial insemination caused a drop in egg production compared with bi-weekly handling and overall fertility was likewise affected. It is possible that stress of any kind may interfere with the transport of spermatozoa in the oviduct with a consequent effect on fertilization rate.

### 2.14.4 The deposition of semen

The distal portion of the vagina must be well everted before semen is inserted into the oviduct. The ease with which this can be done varies among species of domestic birds. The procedure is carried out by first putting pressure on the abdomen of the female, and then releasing it allowing the oviduct to

resume a normal position before actually ejecting the semen from the cannula or syringe (Lake and Stewart, 1978). Lake *et al.* (1985) reported success after semen of fowl deposited at 5cm depth similarly optimal fertilization was obtained with the depth of 0.5cm of the vagina opening.

## 2.15 Fertility in poultry

Fertility can be defined as the percentage of eggs laid that are fertilized (Singh, 1990). Fertility in poultry depends on production of viable eggs from the female and viable sperm from the male which fuses to form a fertile egg. The male sperm attaches to the perivitalline membrane and makes a whole as it enters the egg. Hundred of spermatozoa's may enter the yolk but the fact is that, the more likely the egg will be fertilized. To ensure 95 percent chance of fertilization, about 30 sperm cells must enter the egg near the germinal disc.

Although it is true that only one sperm is necessary to fertilize an egg, the probability of an egg being fertilized by only one sperm reaching and penetrating it is very low (Mc Daniel, 1993).

In turkeys high fertility is obtained 20 days after insemination. It then decreases slowly with some fertile eggs obtained 10 weeks after insemination (Lorenz, 1959). One insemination will provide spermatozoa to fertilize some eggs for many weeks. However, for maximum fertility, insemination will be 3 inseminations in the first 7 days, or 4 in 12 days. The second insemination should be carried out two days after the first and the third being not later than the end of the first week's production period. A double insemination (Two

within seven days) can be beneficial to help maintain high fertility (Tech. Advice sheet, 2006).

The percentage of eggs laid can be affected by several factors namely:- Male to female ratio, Nutrition, Age of bird, Sperm quality, Breed differences and Temperature.

### 2.15.1 Fertility period of turkey

From a biological point of view, turkey remains for 3 to 4weeks after one insemination of 80 and 100million spermatozoa. However, once weekly insemination are common in practice. In an interesting paradox, while turkey sperm stored in-vitro for 24hours lose functional competence, turkey sperm residing in hen's sperm storage tubules (SST) for a long period of 16weeks induced fertility of eggs. It is also hypothesized that prolonged storage in the SSTs is supported by reversible suppression of metabolism and motility and/or stabilization of the plasma membrane and maintenance of the acrosome (Bakst *et al.,* 1994).

## 2.16 Hatchability in poultry

Hatchability refers to the proportion of fertile eggs that are set for incubation either artificially or naturally that hatched into viable chicks. Hatchability is closely related to the level of fertility, thus an egg must be fertile before it could hatch. Hatchability is a reflection of the level of embryonic maturity during the period of incubation (Nwosu, 1999). Fertility and

hatchability are the most important determinants of the number of chicks from a given number of breeding stock within a stipulated period.

Keeping eggs for more than 10 days reduces hatchability. However, chicken and turkey eggs are an exception. Chicken eggs have been stored for 3 weeks to 4 weeks without appreciable loss in hatchability (TAES 2006).Hatchability is best maintained by storing eggs with the small end down in sealed, and air tight plastic bags. Hatchability is affected independently by a number of factors such as storage and handling practices, fertility of eggs, incubator temperature, incubator humidity, incubator ventilation, egg turning/setting and egg porosity. Hatchability can never be better than fertility (Mc Daniel, 1993).

# CHAPTER THREE

# MATERIALS AND METHODS

## 3.1 Location and duration of experiment

The Experiment was carried out at the poultry unit of the Department of Animal Science, University of Nigeria, Nsukka.

The Farm is of fairly gentle slope (0.2%) and it falls within the humid equatorial tropical climate generally known for its well defined rainy seasons (April-October) and dry seasons (November-March). Annual rainfall ranges from 1680mm -1700mm (Breinholt *et a*l., 1981). Nsukka lies in the Dried Savannah region, and is located on Longitude $6^0$25'N and Latitude $7^0$24'N (Ofomata, 1975) at an altitude of 430m above sea level (Breinholt *et al.*1981).The climate is a typical humid setting with RH range of 56.01 - 103.83%.Average diurnal minimum temperature ranges from $22^0$C -$24.7^0$C while the average maximum temperature ranges between $33^0$C -$37^0$C (Okonkwo and Akubuo, 2007), Energy Centre, UNN, 2008).

### Duration of the study

The work lasted for a period of ten (10) weeks preceding two weeks pre-experimental period.

## 3.2 Experimental birds

A total of fifty-one (51) local turkeys of Broad Breasted Bronze breed, aged approximately 8 months comprising of six (6) toms and forty- five (45)

hens were procured from Turkey Market in Ekwulobia, Anambra State for the study.

### 3.3 Management of birds

The birds were reared on deep litter, fed breeder's ration (containing 17% crude protein) and given water *ad-libitum.* Vaccines and medication were administered at the appropriate times to avoid incidence of diseases.

#### 3.3.1 Allocation of experimental birds into treatments

Birds were housed together until they attained sexual maturity or first point of lay. Thereafter both toms and hens were separated. One (1) tom was housed per pen measuring 5ft x 5ft as shown in Table 6 below while three hens were housed per pen measuring 5ft x5ft as shown in Table 7. Individuals in each group were fitted with numbered leg bands for individual identification.

**Table 6: Distribution experimental birds (Toms)**

| **T1** | **T2** | **T3** | **T4** | **T5** | **T6** |
|---|---|---|---|---|---|
| 1 | 1 | 1 | 1 | 1 | 1 |

**Table 7: The distribution of experimental birds (Hens) among treatments**

| | **TREATMENT (BIRDS)** | | | | |
|---|---|---|---|---|---|
| **Dosage(ml)** | **D0** | **D1** | **DII** | **DIII** | **DIV** |
| **0.2** | 3 | 3 | 3 | 3 | 3 |
| **0.3** | 3 | 3 | 3 | 3 | 3 |
| **0.5** | 3 | 3 | 3 | 3 | 3 |
| **Total** | **9** | **9** | **9** | **9** | **9** |

### 3.4 Semen collection

Semen samples were collected from each tom twice a weak (i.e. Tuesdays and Fridays by dorso-abdominal massage as described by Burrow and Quinn (1937) and pooled to eliminate the effect of individual variability of gamete donors. Care was taken to avoid contamination of semen with cloaca / fluids. Collection of semen was done by stimulating the copulatory organ to protrude by massaging the abdomen and the back over the testes. This was followed by pushing the tail forward with one hand and at the same time using thumb and fore fingers of the same hand to milk semen from the duct of this organ. The semen was collected in a glass cylinder. A total of one hundred and fifty semen (150) samples and eighteen (18) semen pooled samples with insemination dose of $200x10^6$ were used.

### 3.5 Semen dilution and evaluation of sperm survivability

The pooled semen were mixed and split into five (5) samples of equal volume. Thereafter aliquots of semen extracted from each split was diluted in different diluents to constitute Diluents: DI, DII, DIII and DIV. DO (neat semen) was the control. Each of the diluents was made up as shown in Table 8. The dilution rate adopted with each of the test diluents was 1:1.Thereafter the mixture of semen and medium was stored at room temperature for evaluation and insemination. Different semen preservation tubes labeled DI, DII, DIII, DIV and DO were used for the experiment.

**Parameters measured**

**(a) Determination of semen quality in the laboratory or laboratory evaluation of semen**

The following semen characteristics were determined in the male:

### 3.5.1 Semen volume (ml)

The volume of ejaculate of each tom was determined from the caliberated glass collection tubes immediately after collection; volume of ejaculate was recorded in ml.

### 3.5.2 Color and consistency

These were determined by visual appraisal to ascertain the color and consistency.

### 3.5.3 Sperm concentration

This was determined by counting spermatozoa using haemocytometer. A haemocytometer was used to determine sperm concentrations of the semen samples. A 1:100 dilution of the semen was prepared by mixing 10ml of semen with 990uml of 2% (wt/vol.) acetic acid. Both chambers of the haemocytometer were filled with the dilute semen sample and allowed to settle for 3mins. Using a 400X magnification, the numbers of sperm cells in five large squares at diagonal of the chamber were counted. The values were recorded in ($x10^9$/ml).

### 3.5.4 Sperm motility

A drop of semen was placed on a warm glass slide and mixed with equal drop of 0.9% of the formulated diluent. The mixture was covered with cover

slip and examined under the microscope (x400). Progressive motility of sperm was scored on a scale of 0-100. The slides and cover slips were warmed to $37^{O}C$ to minimize cold shock on sperm. The values were recorded in percentage.

### 3.5.5 Sperm pH

This was measured using pH meter. The range of 7.5 was maintained for the different diluents used.

### 3.5.6 Sperm morphology

Stained slide was viewed at x1000 magnification and 100 sperm evaluated. Morphological percent normal and abnormal spermatozoa were evaluated.

## 3.6 The Composition of the different diluents are presented in Table 8

**Table 8: Composition of the different Diluents**

| | QUANTITY | | | | |
|---|---|---|---|---|---|
| **INGREDIENTS** | D0 | D1 | DII | DIII | DIV |
| Sodium chloride(g) | - | 9.5 | 9.5 | 9.5 | 9.5 |
| Potassium chloride(g) | - | 0.2 | 0.2 | 0.2 | 0.2 |
| Calcium chloride(g) | - | 0.26 | 0.26 | 0.26 | 0.26 |
| Sodium bicarbonate(g) | - | 0.2 | 0.2 | 0.2 | 0.2 |
| Distilled water (ltr/350m.osm7.5 | - | 1 | 1 | 1 | 1 |
| Glucose(g) | - | - | 1 | - | - |
| Coconut milk(ml) | - | - | - | -1 | - |
| Orange juice(ml) | - | - | - | - | 1 |
| Antibiotic | - | - | - | - | - |

*0-Control-Raw Semen
*1--Ringers Solutions
*I1-Ringers solution + Glucose
*III-Ringers solution + Coconut milk
*IV-Ringers solution + Orange juice

The following parameters were evaluated in two phases with the stored semen

### 3.7 Phase I: Survivability rating

The motility of semen diluted as indicated in Table 8 was evaluated carefully every hour as indicated earlier to determine the survivability status of sperm in the various media until the sperm reached senescence. The motility ratings were done and recorded in percentages (%).

### 3.8 Phase II: Insemination

Insemination began after each bird had laid the first egg. Each bird was monitored daily for onset of egg laying, which opened the reproductive tract and facilitated insertion of the insemination syringe. Each hen was caught manually. The abdomen was gently palpated to ensure the absence of a shell covered egg in the oviduct that could prevent the sperm from reaching the site of fertilization. Three doses of each diluted semen (0.2, 0.3 and 0.5ml) were respectively deposited into the reproductive tract of the three hens allocated to each experimental unit as indicated in Table 7 using the insemination syringe. Two persons were involved in the insemination; one held the hen upside down and palpated the abdomen to expose the reproductive tract while the other deposited the semen through the opening of the reproductive tract at the cloaca. Insemination of hens within each group was done twice a week according to the experimental plan for six weeks. All insemination was done in the afternoon between 2.00 p.m to 3.00 p.m.

### 3.9 Egg collection, storage setting in the incubator and candling

Eggs were collected daily using egg creates, labeled and stored in a room at temperature range of 25 – 28$^0$C and relative humidity of 75%. On the 7$^{th}$ day, they were set in the incubator at Uchecen Hatching Centre, No .39A Justina Street, Odenigbo- Nsukka. Thus, eggs collected were set in the incubator every seven (7) days. Seven (7) days after the start of incubation, candling was done to check evidence of embryonic development/fertility. At 28 days after incubation, hatching rates of fertile eggs were assessed and the formulae shown below were applied to determine percent fertility and hatchability of eggs in each experimental unit.

(i) $$\%\,\text{Fertility} = \frac{\text{Fertile egg}}{\textit{Egg set}} \text{X } 100$$

(ii) $$\%\,\text{Hatchability} = \frac{\text{Chicks hatched}}{\textit{Fertile eggs}} \text{X } 100$$

### 3.10 Experimental design

The experimental design used in the experiment was a 3 x 5 factorial arrangement in a completely Randomised design using the following linear model:

$X_{ijK} = \mu + A_i + B_j + (AB)_{ij} + E_{ijK}$

Where Ai = Effects of diluents on the ith group

Bj = Effects of semen dosage on the kth group

(AB)ij=Effects of interaction between diluent and dosage rate

Eijk = Effect of random error

**Statistical Analysis**

All data obtained were subjected to factorial analysis of variance (Factorial ANOVA). The means, standard errors of the means were calculated for parameter using (SPSS 2001) and significant difference between means were separated using Duncan's New Multiple Range Test (DNMRT), (Duncan, 1955).

# CHAPTER FOUR

# RESULTS AND DISCUSSION

## 4.1 Semen characteristics of toms used in ambient temperature semen storage

The semen characteristics of six (6) different toms used for the study is presented in Table 9 below.

**Table 9: Semen quality of toms used in ambient temperature semen storage**

| | TREATMENT(toms) | | | | | | |
|---|---|---|---|---|---|---|---|
| **PARAMETER** | **T1** | **T2** | **T3** | **T4** | **T5** | **T6** | **OVERALL** |
| Volume(ml) | 0.21± 0.02 | 0.23 ± 0.25 | 0.23± .27 | 0.03± 0.04 | 0.17± 0.02 | 0.19± 0.00 | 0.21± 0.01$^{NS}$ |
| Motility(%) | 81.95±3.19 | 80.80± 0.93 | 82.53±1.19 | 81.40±0.48 | 82.75±0.55 | 80.00±0.76 | 81.57± 0.58$^{NS}$ |
| Concentration ($x10^{9/ml}$) | 3.37±1.60$^{b}$ | 3.34± 1.30$^{b}$ | 3.38±0.94$^{b}$ | 3.30±0.84$^{b}$ | 3.57±1.37$^{a}$ | 3.57±1.37$^{a}$ | 3.42±1.24** |
| Abnormality (%) | 18.30±3.03 | 19.20±0.93 | 17.55±1.19 | 18.60±0.48 | 20.00±0.76 | 20.00±0.76 | 18.41±.59$^{NS}$ |

a,b means with different superscript are significant at 1%
$T_1 – T_6$ = Individual toms used
NS = Not significant (P>0.05)
** = Highly significant (P<0.01)

The import of this was to ascertain true semen quality of each tom before using them for the storage and insemination trials so that unproductive males (toms) would not be used. Statistical analysis showed no significant (P>0.05) differences among toms in semen volume. However, relatively higher ejaculate volumes were produced by T2 (0.23+0.24ml) and T3 (0.23+0.27ml) toms. Sperm motility and morphologic abnormalities were also found to be respectively similar (P>0.05) among toms. Sperm concentration differed highly

significantly ($P<0.01$) among experimental toms. T5 and T6 recorded similar values for sperm concentration which were higher compared to other toms.

The values for sperm concentration ($x10^9$) in the two treatments were 3.57+1.37 respectively while the lowest value recorded by T4 was 3.30+0.84.

The differences in values of semen quality traits obtained from different toms especially in sperm concentration indicated that individual toms have different reproductive capacities (Benoff *et al.*, 1981) .The semen characteristics of Toms used in the study are within the ranges of values reported in literature for healthy turkey toms ( Zarahdeen *et al,* 2005; Alkan *et al.*, 2010). This is an indication that the toms were normal and devoid of any reproductive defects and are qualified for use in the subsequent ambient temperature storage and insemination studies.

## 4.2 Main effects of age (length of semen storage; hours) on sperm survivability as determined by motility

Result of the effect of age on sperm survivability determined by hourly evaluation of motility scores in stored samples are presented in Table 10.

**Table 10: The effect of Age of stored tom semen on sperm survivability**

| Survivability(hrs) | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | SEM |
|---|---|---|---|---|---|---|---|---|---|
| Motility (%) | $80.47^{a}$ | $72.23^{b}$ | $62.80^{c}$ | $29.83^{d}$ | $21.30^{e}$ | $16.30^{f}$ | $4.67^{g}$ | $0.93^{h}$ | 1.05** |

a – h; Means with different superscript on the same row are significant different at 1% (** - P<0.01).

Table 10 shows the effect of age of stored tom semen on turkey sperm survivability. From the results highly significant differences (P<0.01) were observed among rate of survival of sperm stored from 1 to 8 hours under ambient condition. The highest motility value (80.47%) was recorded at 1$^{st}$ hour while the lowest survival rate (0.93%) was observed at the 8$^{th}$ hour. This implied that motility of sperm decreased with increasing age of stored semen at ambient temperature. This decline in survival of sperm may be as a result of decrease in utilizable substrates including fructose and phospholipid content of spermatozoa during storage. This was corroborated by the report of Douard *et al.* (2000) who reported that motility, viability and morphological integrity of spermatozoa decreased progressively during storage.

## 4.3 Main effect of different diluents on sperm survivability

The effects of different diluents on sperm survivability of tom sperm determined by hourly motility evaluation is presented in Table 11.

**Table 11: Effect of diluents on survivability of toms sperm stored under ambient temperature**

| Parameter | Treatment | | | | | |
|---|---|---|---|---|---|---|
| | $D_0$ | $D_I$ | $D_{II}$ | $D_{III}$ | $D_{IV}$ | SEM |
| Motility (%) | $28.54^{d}$ | $28.04^{d}$ | $41.98^{b}$ | $47.10^{a}$ | $34.67^{c}$ | 0.83** |

a – d; *Means with different superscript on the same row we significant different* (** - P<0.01)

Table 11 presents the effect of diluents on tom sperm survivability after storage from 1-8 hours. There were highly significant (P<0.01) differences in rates of survival of sperm during the 8 hour storage duration showed that diluent DIII gave the highest survival rate of 47.10% followed by diluent DII in which the survival rate was 41.98%.The control (D0) and DI were similar and had the lowes*t* survival rates of 28.54% and 28.04% respectively while DIV exhibited a medium rate of survival (34.67%) when all the diluents were considered .The trend exhibited here is indicative of the fact that turkey semen needs enabling storage conditions to survive (Gadea, 2003) after ejaculation under ambient conditions and diluents DIII,DII and to a lesser extent DIV appeared to possess these condition which most likely are effective buffering capacity of medium and substantial availability of metabolizable substrate. The diluents having these qualities sustained the sperm stored in them more than the

control and DII which lacked these qualities. It seems apparent that if the microbial action was controlled with inclusion of antibiotics in the media, the survival rates may have been higher in the various media. The results also showed that the inclusion of coconut milk to the diluent (DIII) and glucose to (DII) may have resulted in the high sperm survival rates obtained in the two diluents, while the inclusion of orange juice produced better effect than pure ringer solution (DI).

## 4.4 Interaction effect of different diluents and age of turkey semen on the rate of survival (%)

The effects of Diluents x Age of semen on survivability of sperm determined by evaluation of motility on hourly basis are presented in Table 12.

**Table12: Interaction effect of diluent and storage age of turkey semen on sperm survivability**

| Age(hr) | Diluents | | | | | |
|---|---|---|---|---|---|---|
| | **D0 Mean ±SEM** | **D1 Mean ± SEM** | **DII Mean ± SEM** | **DIII Mean ± SEM** | **DIV Mean ± SEM** | **Overall** |
| 1 | 83.83± 2.39 | 72.67±1.15 | 80.00± 4.87 | 82.00± 3.66 | 83.83± 3.27 | 80.47± 1.58$^{NS}$ |
| 2 | 75.83± 1.38$^{a}$ | 66.33±1.73$^{b}$ | 71.00± 1.18$^{ab}$ | 75.33± 2.23$^{a}$ | 72.67± 2.62$^{a}$ | 72.23± 1.01* |
| 3 | 64.83± 2.27 | 57.00± 3.12 | 63.00± 1.63 | 63.33± 2.39 | 65.83± 1.64 | 62.80± 1.11$^{NS}$ |
| 4 | 3.83±.60$^{c}$ | 23.67± 6.94$^{b}$ | 45.83± 6.85$^{a}$ | 53.83± 1.85$^{a}$ | 22.00± 5.19$^{b}$ | 29.83± 3.75** |
| 5 | 0.00±0.00$^{d}$ | 4.67±1.05$^{c}$ | 41.17± 4.88$^{a}$ | 42.50± 1.95$^{a}$ | 18.17± 1.96$^{b}$ | 21.30± 3.37** |
| 6 | .00±0.00$^{d}$ | 0.00±0.00$^{d}$ | 27.33± 2.68$^{b}$ | 39.33± 2.88$^{a}$ | 14.83± 1.14$^{c}$ | 16.30± 2.96** |
| 7 | 0.00±0.00$^{c}$ | 0.00±0.00$^{c}$ | 6.50± 1.88$^{b}$ | 16.3± 1.28$^{a}$ | 0.00±0.00$^{c}$ | 4.67± 1.29** |
| 8 | 000±0.00$^{b}$ | 0.00±0.00$^{b}$ | 1..00± 2.45$^{b}$ | 3.67± 1.51$^{a}$ | 0.00±0.00$^{c}$ | .93± 1.87** |

a,b,c means with different superscript are significant (P<0.01)
* = significant (P<0.05)
** = Highly significant (P<0.01)
NS = Not significant (P>0.05)
Age = Number of hours sperm is kept in diluents at ambient temperature

Significant (P<0.05) differences were observed between diluents (treatments) in the rates of survivability (%) of turkey sperm in the second hour of storage while highly significant (P<0.05) differences occurred among treatments (Diluents x Age of semen in the 2$^{nd}$ and 4$^{th}$ to 8$^{th}$ hours of storage

under ambient condition. The result showed that within 1hour of storage, all diluents maintained high motility that compared favorably with the control with only DI exhibiting more drop in sperm motility (72.67%) compared to other trial diluents and the control (D0) where motility ranged from 80%-83%.At the 2nd hour of storage, high motility of semen was still maintained with DIII sustaining the highest sperm motility value of 75.83±1.38 and DI recording the lowest 66.33±1.73%. The motility value for the control was 75.83% which was statistically similar with value obtained for DII, DIII and DIV. The result of motility score in this period showed significant differences ($P<0.05$) between treatments. This agrees with the result of work by Morel *et al.* (2009) who obtained a related trend in motility value of sperm.

At the 3rd hour motility values of sperm in all extenders were not significantly ($P>0.05$) different. The values ranged from 57.00% in DII to 65.83% in DIV. These motility values were all within the range reported in literature as normal for effective fertility in turkey (Moss *et al.,* 1970).

At the 4th hour DIII recorded the highest value of 53.83+1.85% while the motility of D0 dropped sharply to 3.83+0.60% as the lowest. At this point, the motion of most sperm cells in D0, DI and DIV, were sluggish. There were therefore highly significant differences ($P<0.01$) among treatments in motility values.

At the 5th hour of storage, there were highly significant ($P<0.05$) differences among treatments in sperm motility.D0 sperm exhibited complete loss of

motility at this time while DII and DIII values which were statistically similar (41.17% and 42.50% respectively) and higher than values for other treatments which ranged from 0.0% in D0 to 18.17% in DIV.

At the 8$^{th}$ hour DIII recorded few surviving sperm cells with motility rate of 3.67$\pm$0.62 while DII had the lowest (1.00$\pm$2.45%). Virtually, all the surviving cells were weak. Highly significantly differences ($P<0.01$) occurred among the treatments at the 8$^{th}$ hour of storage.

The findings from this study showed that the survivability of sperm in the various diluents differed. For instance, DII and DIII maintained sperm survivability longer than other treatments. Also D0 could not sustain sperm survivability for up to 5hours and 6 hours respectively.

The longevity of sperm experienced in D0 (neat semen) in this study counters the earlier report by Free patent sloine.com (2009) that turkey spermatozoa usually perishes minutes outside its own body or its own seminal fluid. Notably, the survival of sperm cells in DIII for up to 8hour in the diluent used appears unique especially under ambient conditions. It maintained a steady sustenance of sperm motility/trend throughout the study without complete loss of viability as the case in other diluents .This is probably because of the cytokinin and other chemical components like sugar, vitamin, minerals and amino-acid in coconut which are known to enhance metabolic activities of sperms (Jean *et al.,* 2009).In a like manner the result of this study agrees with the report of Norman (1962) that coconut extender encourages livability of

sperm. The results obtained corroborate the report of Taner *et al.* (2011) that coconut based extenders is a better sperm preservative than Ringer's solution. One of the factors that might have led to the rapid decline of the sperm motility in diluted samples was the non addition of antibiotics to the extender .This probably allowed quick depletion of metabolizable sustrate by the competing microbes.

The lower motility found in sperm stored in ringer solution than sperm stored in glucose extender conforms to the report of Taner *et al*. (2010).

## 4.5 Main effect of different insemination dose on fertility and hatchability of turkey eggs

The results on the effects of insemination dose on fertility and hatchability of turkey semen stored in various diluents under ambient conditions are presented in Table 13.

**Table 13: Effect of different insemination dosage on fertility and hatchability of Turkey eggs**

| **Parameter** | **Dosage (ml)** | | | |
|---|---|---|---|---|
| | **0.2** | **0.3** | **0.5** | **SEM** |
| Fertile egg (%) | 43.32 | 44.23 | 44.32 | $1.92^{NS}$ |
| No of Chick hatched (%) | $54.14^{b}$ | $62.84^{a}$ | $65.57^{a}$ | 3.07* |

a,b – Means with different superscript are significant at (* P<0.05)
NS = Not significant

The result revealed a non significant effect (P>0.05) of insemination dose on fertility of eggs. Highest number of fertility was recorded at 0.5ml with 44.32% and the lowest at 0.2ml with 43.32%. The effect of insemination dose on chicks hatched was significant (P<0.05). Insemination dose of 0.3ml (62.84%) and 0.5ml (65.57%) were similar (P>0.05) in terms of hatchability. However, they differed from hatchability percentage of 54.14 recorded at 0.2ml dosage. This work agrees with the report of Merck & Co (2008) who reported related dosages to achieve fertility results.

## 4.6 Main effect of different diluents on fertility and hatchability of turkey eggs

The results on the effect of different diluents on fertility and hatchability of turkey eggs are presented in Table 14.

**Table 14: The Effect of diluents on fertility and hatchability of turkey eggs**

| **Parameter** | **Diluents** | | | | | |
|---|---|---|---|---|---|---|
| | **$D_0$** | **$D_I$** | **$D_{II}$** | **$D_{III}$** | **$D_{IV}$** | **SEM** |
| Fertile egg (%) | $58.16^{a}$ | $29.99^{c}$ | $40.35^{b}$ | $60.55^{a}$ | $30.76^{c}$ | 2.47** |
| No of Chick hatched (%) | $57.45^{ab}$ | $65.99^{a}$ | $64.09^{a}$ | $65.47^{a}$ | $51.15^{b}$ | 3.96* |

a, b, c, Means with different superscript are significant at (** P < 0.01) (* P < 0.05)

The results showed that the effect of the diluents on proportion of fertile eggs was highly significant (P<0.01) with DIII having the highest fertility value of 60.55%.The fertility value of DIII extender was statistically similar with that of the control (58.16%) while values for DI (29.99%) and DIV (30.76%) were also similar but significantly lower than other values. The number of chicks hatched were also affected significantly (P<0.05) by the diluents with hatchability values of DI, DII and DIII being statistically similar but higher than hatchability values of control (57.45%) and use of DI, DII and DIII in the field could cause improvements in hatchability of eggs probably due to their balance in metabolizable substrates and buffering capacity (Norman, 1962; Reuden *et al.,* 1981).

## 4.7 The interaction effect of Diluents and Insemination dosage on fertility of Turkey eggs

The result of the interaction effect of diluents and insemination dosage on fertility of turkey is presented in Table 15 below

**Table 15: Interaction effects of diluents and dosage on fertility of Turkey eggs**

| Dosage (ml) | Treatment(diluents) | | | | | |
|---|---|---|---|---|---|---|
| | **D0** | **DI** | **DII** | **DIII** | **DIV** | **OVERALL** |
| | **Mean ± SE** | **Mean ± SE** | **Mean ± SE** | **Mean ± SE** | **Mean ± SE** | |
| 0.2 | 58.90± 6.72$^{a}$ | 31.10± 2.83$^{b}$ | 38.86± 2.48$^{b}$ | 56.66± 3.25$^{a}$ | 31.10± 2.83$^{b}$ | 43.32± .96** |
| 0.3 | 58.90± 7.38$^{a}$ | 31.08± 2.22$^{b}$ | 39.98± 2.07$^{b}$ | 62.24± 7.54$^{a}$ | 28.96± 2.12$^{b}$ | 44.23± .49** |
| 0.5 | 56.69± 4.46$^{a}$ | 27.78± 5.83$^{c}$ | 42.20± 2.83$^{b}$ | 40.35± 1.38$^{a}$ | 60.55± 2.75$^{bc}$ | 43.96± 1.83** |

a, b, bc ,c:Means with different superscript are significantly different at (P<0.0.01)
** = Highly significantly (P<0.01)

The interaction effects of diluents and dosage on fertility of turkey eggs was highly significant (P<0.01).Thus, there was variation in the fertility of turkey eggs at different dosages and diluents. For example, the highest fertility value of 62.24±7.54% was recorded for DIII at 0.3ml dosage while at insemination doses of 0.2 and 0.5ml, the fertility values were 56.66% and 40.35%. DIV had its highest fertility value of (60.55% with 0.5ml dose while fertility values at 0.2 and 0.3ml doses were 31.1% and 28.96% respectively. DII had its highest fertility value of 42% with insemination dose of 0.5ml while fertility values at 0.2 and 0.3 dosages were 38.86% and 39.98% respectively. Fertility rates at various doses with DI diluent were low 31.1%, 31.08% and

27.78% with insemination doses of 0.2ml, 0.3ml and 0.5ml respectively. Only the control had consistent and similar values of 58.9%, 58.9% and 56.69% respectively.

It does appear that relative to control, there was significant diluent x dose influence on turkey fertility with the best being DIII at 0.3ml dose and DIV at 0.5ml dose .Interaction effects between DI and DII with the various insemination doses produced low fertility results. It seems apparent that for field application, DIII diluents gave best results at 0.3ml insemination dose while DIV gave best fertility results at 0.5ml insemination dose. Raw semen (control) can successfully be used at 0.2ml, 0.3ml and 0.5ml respectively with good fertility results.

## 4.8 Interaction effects of diluents and dosage on hatchability

The effects of Diluents x Insemination dosage on hatchability is presented in Table 16.

**Table 16: Effect of diluents and dosage on hatchability**

| | Treatment (diluents) | | | | | |
|---|---|---|---|---|---|---|
| **Dosages (ML)** | **D0 Means ± SEM** | **D1 Means ± SEM** | **DII Means ± SEM** | **DIII Means ± SEM** | **DIV Means ± SEM** | **Overall** |
| 0.2 | 50.56± 3.41 | 63.48± 8.28 | 59.74± 8.91 | 57.88± 5.67 | 39.04± 4.10 | 54.14± 3.17$^{NS}$ |
| 0.3 | 56.92± 8.15 | 71.66± 6.23 | 66.54± 3.17 | 63.42± 2.87 | 55.66± 9.33 | 62.84± 2.92$^{NS}$ |
| 0.5 | 64.88± 5.93 | 62.82± 10.80 | 65.980± 8.09 | 75.12± 4.61 | 58.76± 6.88 | 65.51± 3.28$^{NS}$ |

NS = Not significant (P>0.05)

The results generally indicate no significant (P>0.05) interaction effect of diluent and insemination dose on hatchability of Turkey eggs. However, the hatchability results of eggs laid by hens inseminated with semen stored in the various diluents using the various doses were generally above average and ranged from 55.66% with DIV at 0.3ml insemination dose to 75% obtained for DIII at 0.5ml insemination dose. It is also important to indicate that the dilution rate of 1:1 adopted for all the diluents yielded desirable results which were consistent with the results reported by Long (2003).

## Summary and Conclusion

The present study has shown that diluents containing coconut milk and orange juice yielded relatively high sperm survivability as was evident with the motility, fertility and hatchability results obtained. This observation is made out of the highly significant interaction effects obtained for survivability, fertility and hatchability of eggs when these diluents were used. It appeared evident that the constitution of the diluents in term of buffering and stability of metabolizable substrate were adequate for turkey semen. The coconut milk had a slight edge over the orange juice in view of the results obtained and showed better balance of natural constituents that favor sperm viability and fertility even under ambient condition. There is need for further investigation of these diluents and certainly with inclusion of antibiotics in order to make more concrete statements about them for field use.

**Recommendation**

Based on the results presented it is recommended that:

1. The use of artificial insemination should be encouraged and stepped down to every turkey farmers to avoid loss of genetic potentials in toms which are recalcitrant to mating due to heavy weight.
2. There is need for in depth study of semen diluents/extenders to further highlight more information on turkey semen preservation. Similarly improvement should be targeted at proper exploitation of local

metabolizable substrates like coconut milk and orange juice to minimize cost.

3. There is need to invest heavily on improvement of local turkeys to provide sufficient protein source as well as save our turkey industry from decay.
4. To achieve higher fertility and hatchability rates, adoption of good management practices under good environment is eminent. This calls for proper installation of incubators and hatching machines in the poultry farm to take care of poults.

## REFERENCES


Ademosum, O. (1986). Sociological survey of Salmonella pullorum antibody in chickens around Zaria. Proceed NSAP, 2008.

Ajala, M. K; Nwangu, B. I; Sekoni, A.A and Adesehinwa, A.O.K. (2007).The profitability of turkey production in Zaria, Kaduna state Nigeria. *Asian Journal of Information Technology* 6(1):27-33.

Akcay, E. and Tanner, H. (2009). Fertilizing ability of short-term Preserved spermatozoa Abant trout.

Akcay, E.; Varisli, O. and Tekiin, N. (2009). Fertilizing capacity of Turkey semen diluted with simple sugar-based extenders after cooled storage. Internet.www.Goggle.com-aklay@vet.

Alavi-Shoutari, S.M.; Ahmadi, M.; Shahvarpour, R. and Kolahian, S. (2007). Effect of Tiamullin, Neomycin, Tetracycline, Fluorophenicol, Penicillin G., Linco-Spectin, Erythromycin and Oxytetracycline on controlling bacterial contaminations of the River Buffalo *(Bubalis bubalis)* semen. *Pakistan Journal of Bio. Sc*., 10 (18):3200-3204.

Alkan, C., Sydamant, P. Kitzman, J. O., Antonenali, G. (2010). Molecular Biology and Genetics-Diversity of human copy number Variation and Multicopy-*mbg.cornell.edu*>MBG>Reseach>Clark.Lab.PDF(1):195-198.

AX, RL; Dally, MR; Didon, BA; Lenz, RW; Love, CC; Varner, D. D; Hafez, B. and Belline, M. E. (2000).Artificial Insemination. *Iranian Journal of Veterinary Research*,Vol.7.No.4 Ser.No.17,2006.

Bakst, M.R. (1980). Fertilizing Capacity and Morphology of Fowl and Turkey spermatozoa in hypotonic extender. *J. Reprod. Fert*., 60:121-127. [Abstract/Free].

Bakst, M.R. (1981). Sperm recovery from oviducts of turkeys at known intervals after insemination and oviposition. *J. Rerod. Fert.*, 62:159-164.

Bakst, M.R. and Cecil, H.C. (1997). Techniques for semen evaluation, semen storage and fertility determination. *Poult. Sc. Ass. Savoy II*

Bakst, M.R; Wishart, G. and Brillard, J.P. (1994). Oviductal Sperm selection, transport and storage in poultry. *Poultry Sci. Reviews*, 5, 117-143.

Barbara SR.; Michal, B. and Zdzislan, S. (2003) Effect of Antioxidants added to boar semen extender on the semen survival time and Sperm chromatin structure.

Benoff, F.H; May, E.B. and Renden (1981). Histochemistry of uterovaginal sperm-host glands in Japanese quail (Coturnix coturnix japonica) with reference to the period of oviposition. *Poultry Science* 60, 2529-2535.

Blesbois, E. and Brillard, J.P. (2007). Specific features of in vivo and in vitro sperm storage in birds.1.10pp 1471-1481.Animal consortium.

Bonadona J.; Fornarcoli, D. and Pozzi, G.G. (1962). The Utilization of some vegetable juices for the dilution of semen.*Zootech and Vet*.116-118

Bonadonna, T. and Succi, G. (1980). Artificial Insemination in the world .Proc.ix int. Conf. Anim. Reprod. Artif. Insem. Madrid.

Breinholt, K. AI.; Gowen, F.A and Nwosu, C.C. (1981). Influence of Environmental and Animal Factors on Day and Night Grazing Activity of Imported Hostein-Frezian Cows in the Humid Lowland Tropics of Nigeria. *Trop.Anim.Prod*.6:4.

Brillard, J. P; Beaumont, C. and Scheller, M.F. (1998). Physiological responses of hen divergently selected from a single insemination. *Journal of Reproduction and fertility*, 114,111-117.

Brown, K. I.; Nestor, K. E and Topscher, M. (1970). A comparison of various methods of estimating spermatozoa concentration of turkey semen. *Poultry Sci.*, 49:295-299

Bunacui, P.; Muscalu, G.; Bunacui, M.; Soerban, D.; Penalt, M. (1990). Hybridazation between muscovy drakes and pekin ducks using artificial inseminations.

Burke, W.H. (1984). Avian reproduction. In: Dukes physiology of Domestic Animals. ($10^{th}$ edn. M. J. Swenson (ed.), Cornel University press, New York, pp.847-862.

Burrows, W. H. and Quinn, J. P. (1937). The collection of spermatozoa from the domestic fowl and turkey. *Poult. Sci*., 16: 19-24.

Carmen, R.P and George, J.M. (2004). Poultry meat and egg production-CBS Publishers. 4596/1 A.

Carrazoni, J.A. and Bojanovich, O. (1973) Artificial insemination with semen diluted with coconut milk and stored at room temperature. Gaceter Veterinarian, 35(72) Anim-Breed.Abstract, 42.p.551 No.4818.

Central Poultry Development Organisation (2009). *Turkey management guide*. Bangalore, India.pp.2-3

Cerolini, S.; Kelso, K.A.; Noble, R. C.; Speake, B. K.; Pizzi, F. and Cavalchini, L.G. (1997). *Relationship between spermatozoan, composition and fertility during aging of chickens-Biology of Rep*.Vol.57,976-980.

Cerolini, S.; Maldjian, A.; Pizzi, F. and Giozzi, T.M. (2001). Change in quality and liquid composition during cryopreservation of boar semen-*Research*, 121,395-401.

Charlotte, L. (2011) Ingredients of the week: Butternut Squash. www.charlotte.locavore.com

Christense, V.L. and Johnson, N.P. (1975). The effect of time of day of insemination and oviposition on the fertility of turkey hens. Poultry Sc.54:1209.

Cupps, T. P. (1991). *Reproduction in Domestic Animal*, 4th ed. Pg. 11.

Deaton, J.W.; Reece, F.N and Nauhton, F.C. (1978). The effect of temperature during the growing period in broiler performance. *Poult Sc*., 18:715-722.

Donard, V.; Dittermier, and Blesbiosis, E. (2000). Changes in turkey semen lipids during liquid in vitro storage. Biology of Rep. Bio one.

Donard, V.; Hermier, D. and Blesbois, E. (2010). Changes in turkey semen lipid in vitro-storage.

Donoghue, A. M., Garner, D. L., Donoghue, D. J. and Johnson, L. A. (1995). Viability assessment of turkey sperm using fluorescence staining and flow cytometry. *Poult. Sci.*, 74(7): 1191-1200.

Donoghue, A.M. (1998). Prospective Approaches to Avoid Flock Fertility problems: Predictive Assessment of sperm function trials in poultry-USDA,ARS, Germplasm and Gamemete physiology laboratory, Beltsville, Maryland 20705.

Donogue, A. M, Wishart C.I. (2000) Storage of poultry semen. *Anim. Reprod.Sci*.62; 213 -23.

Donogue, A.M. and Walker-simmons (1999). The influence of wheat dehydration-induced proteins in the function of turkey spermatozoa after twenty –four hours in vitro storage. *Poult Sci.*, 78, 235-241.

Douard, V.; Hermier, D. and Blesois, E. (2000) Changes in turkey semen liquids in in vitro storage. Biology of Reproduction 63 1450-1456. [Abstract/Free Full Text]

Dumpala, P. R, H. M. Parker and C.D Mc Daniel (2006). The effect of semen storage Temperature and diluent type on the sperm quality index of broiler breeder Semen. Int.J.Poult.Sci.,5(9).835-845.http:// www.pjbs.org/ijps/fin707.pdf

Energy Research Centre, UNN (2008).Annual Weather Record of Nigeria, Nsukka.

Ensminger, M.E. (1977). *Animal Science (Animal Agriculture Series)*7th edition.

Etches, R. J. (1998). *Reproduction in Poultry*. CAB International Wallingford.

Falcao-e-cunha, L.; Castro-Solla, L.; Maerleus, L.; Marounek, M.; Pinheiro, V.; Freire, J. and Mourao, J.L. (2007). Alternative to antibiotic growth promoters in rabbit feeding. A review *World Rabbit Science* 15:129-140.

FDLPS (1992) *Nigeria Livestock Resources*,Vol:11 National Synthesis.

Foote, R.H. (1978). *Reproduction in farm Animals*.

Foote, R.H. (1980). In "*Reproduction in farm Animals* (E.S.E.,Hafez,ed.)

Foote, R.H. and Trimberger, G.W. (1968). "Artificial Insemination, Reproduction in Farm Animals" . Philadelphia, Lea and Febiger, 135-137.

FreePatentSoline (2001)TurkeySemenExtenderhttp://www.freepatent.com/4329337

Gadea, J. (2003). Semen extender used in the artificial insemination of Swine. *Spanish Journal of Agricultural Research*, 1(2),17-27.

Gadea, J. (2009). Semen extenders used in the artificial insemination of swine, A review-Pig industry-mhmti:file://E :/semen % 20 composition %202-mhtn

Garner, D.L. (1991). *Artificial Insemination. In Reproduction in Domestic Animals. Cupps (Ed) 4th Edition.* New Yolk. Acad. Press. pp 261-262.

Giesen, AF. and Sexton, T. (1983). Belsville poultry semen extender.7.comparison of commercial diluents for holding turkey semen six hours at 15oC-PMID 6835912.

Giesen, A.F.; Mc Daniel, G.R. and Sexton, T.I. (1980). Effects of time of day of Artificial Insemination and oviposition-insemination interval on broiler breeder hens. *Poult Sc.,* 59:2544-2549.

Graham, E. F.; Nelson, D.S. and Schmell, M.K.L. (1982). Development of extender and techniques for frozen turkey semen.1. development. *Poult.Sci,* 61:550-557.

Grunert, J. H.; C. DeGeyter, J. Bortdt, H.P.G Schneider and E. Neischlag (1989). Does Computerised image analysis of sperm movement enhance the predictive value of semen analysis for in-vitro fertilization results? *Int J.Androl*.12:329-338.

Hafez, E. S. E. (1987). *Reproduction in farm Animals*, 5th ed. Pg. 98.

Hafez, E.S.E. (1985). *Reproduction in Farm Animals*. Lea and Febiger, Philadephia. pp. 494-496.

Han, X. F, Z. Y. Niu, F. Z. Liu, Yang, C. S. (2009). Effect of Diluent, Cryoprotectants, Equilibratio Time and Thawing Temperature on Cryopreservation of Duck semen-*Directory of open Access journals*.

Holsberger D.R,A.M .Donogue, D. P. Froman and M.A Ottinger (1998), Assessment of ejaculate quality and sperm characteristics in turkeys: sperm mobility Phenotype is independent of time.*Poult.Sci*;77:1711 PMID:9835348.

Howarth, B. F. (1979). Comparison of extenders for holding cock semen six hours at $41^0$C. *J.Repod.Fertil*.57;149-155 (Abstract/Free Full Text)

HSUS Report (2008). The Welfare of Animals in the Turkey Industry. *http//www.hsus/farm/resources/reseach/welfare.turkeys.htm*

Illori, B.M.; Peters, S.O.; Arubela, D.D.; Akano, K.; Wheto, M.; Iyanda, A.; Ozoja, M.O and Isidahomen C.E (2009). Heat tolerance traits among pure and cross bred Turkey in South West Nigeria. Proceed.14th An. Sc. Conf. pg 89-92.

Javandel, B.; Navidshad, J.; Selfdavati, G.H.P and Baniyaghoub, S. (2008). The Favourite dosage of garlic meal as feed additive in broiler chickens rations. *Pakistan Journal of Biological Science*, 11:1746-1749.

Jean W.H, Yong, Liya Ge, Yan Fei Ng and Swee Ngin Tan (2009). The chemical composition and biological properties of coconut (cocos nucifera l) water-molecules, 14 (51): 44-5164; doi:10:3390/molecule 14125144www.mdpi. com/journal/molecule

Kosin I. I., E.S.T Pierre and Mc Laughlin R. (1951). The prevalence of early Embryonic mortality in Broad Breasted Bronze *Turkeys. Poult Sc*. 30.805-814.

Kosin, L.I. (1968). *Reproduction of poultry. Adopted from Hafez ESE* 2nd edition, pp: 301-319.

Laffaldano, N.; Manchia, A.; Gambacorta, M.; Miachele, D.; Maria, P.R. (2010). Effect of Different Sperm Concentration of Post-thaw viability and motility of Turkey Spermatozoa Cryopreserved by pellet method. *Italian Journal of Animal. Sc*.

Lake, P. and Ravie, O. (1982). Effect on Fertility of Storing turkey semen for 24 hrs at $10^0$C in fluids of different pH.Br. *Poult.Sc*. 23:41-47.

Lake, P. E., Ravie, O. and Waddington, D. (1985). Some effects of the composition of inseminated semen and the site of its deposition on fertility in gallus domesticus, vol. 9, Issue 3, pg. 273 – 284.

Lake, P.E and Stewart, J.M. (1978). Artificial Insemination in Poultry. Ministry of Agriculture, Fisheries and Food, Bulletin No.213. London. Her majesty's stationery office.

Lake, P.E. (1962). In "*The Semen of Animal and Artificial Insemination*" (J.P.Mank ed.).

Lake, P.E. and Ravie, O. (1981) An attempt to improve the fertility of stored fowl semen with certain additives in a basic extender. *Reprod. Nutr. Dev*., 21:1077-1084.Medline.

Leighton A., Caret I., Potter L; Coleman J.and Beane W. (1969). The effect of aeration on the fertilizing ability of turkey semen stored for 48hrs at 5 and $15^0$C. *Reprod. Nutr. Develop*, 24:147-153.

Liu S.J, Zheng J.X and Yang N (2009). Semen quality factor as an indicator of Fertilizing capacity for geese-http://ps:fass,org/cgi/content/full/87/1/155.

LLinskii, E.V.; Ten, E.B.; Tolstov,V.A. and Strelkova, L.I. (1975). Artificial Insemination of caged hens. A.B.A .43(5) no.2048.

Long J.A and Kramer M (2003). Effect of vitamin E on lipid perioxidaton and fertility after artificial insemination with liquid-stored turkey semen. *Poult. Sc*.82,11,1802-1807.

Long, J. (2003). *Poultry semen extender*-Beltsville,MD US,USDA,AKS,OTT

Long, J., Murry R. and Bakst (2008). Biotechnology and Germplasm. Laboratory

Lorenz, F.W.; Abbot, U.K.; Asmondson, V.S.; Adler, H.E.; Kratzer, F.H.; Ogasawara, F.X. and Carson, J.D. (1959). Turkey Fertility. Agric. Ext. Std. Bull.472.

Lukaszewicz, E. (2001). Effects of semen filtration and dilution rate on morphology and fertility of frozen spermatozoa.PMID:114144B.

Machebe, N.S. and Ezekwe, A.G. (2005). Effect of Season on ejaculate quality of three genotypes of Nigeria Local Cocks-Proceed. of the $30^{th}$ Annual Conference of NSAP.Vol.30 pg. 24

Marire B.N (2011) Farm Animals, Features, Husbandry, Health and Production.

Mayer, G.B.; Props, A.T.; Leighton, Jr. H.P.; Vankrey, D. and Potter, L.M (1980). Influence of dietary protein during the pre-breeder period on subsequent reproductive performance in large white turkeys. *Poult Sc*., 59:363-368.

Mc Daniel, C. (2006). Influence on the ability of semen quality index analyzer to predict fertility.

McCartney, M.G and Brown , K.I. (1959). Spermatozoa concentration in three varieties of Turkeys. *Poult Sc.*, 38:390-394.

Merck…Pub,.(2008).Artificialinsemination:Introduction.Disclaimer/Feedback. *www.merckmanuals.con/vet/poultry/ar…*

Morrel J.M; Tjellstrom, H.; Person, B. and Hofmes P.V. (2009) 16 Fertilizing potential of stored turkey sperm. *Journal on Reproduction, Fertility and Development*- File://E:lab.5.htm

Moss, J. A.; Melrose, D. R.; Reed, H. C. B. and Vand de Plassche, M. (1970). Spermatozoa, Semen and Artificial insemination. In: Fertility and Infertility in Domestic Animals, 3rd ed. London: Brailhere, Tindall and Cassel, p. 59-70.

Mounro, S.S. (1938). The effect of dilution and density on the fertilizing capacity of fowl sperm suspensions. *Canad. J. Res. D.*, 16:281-299.

Murray R. Bakst (1993). Future Developments in Artificial Insemination Technology. *J. Appl. poultry Res*. 2:373-377.

Noirault, J. and Brillard, J. P. (1999). Effect of Frequency of semen collection on Quantitative and qualitative characteristic of semen in Turkey Breeder Males.

Norman C.J. (1962). Survival and Fertility of bovine spermatozoa kept at variable temperature in coconut milk extender-*J.Agric.Sc*.59:1803-1807.

Nwosu, C. C. and Mfor, R.A. (1999). Characterization of Local Chicken Strains and Screening of Indiscrete Population of Indigenous Chickens for Production and Reproductive Traits. Local Chickens Project Report 95/73957.University of Nigeria ,Nsukka.

Ofomata, G.E.K. (1975). *Soil erosion .In Nigeria in Maps*. Eastern States. Benin, Ethiopia Pub. House. Pp 43-45.

Okonkwo, W. I. and Akubu, C.O. (2007). Trombe wall system for poultry brooding. *Int. J. Poult. Sc*., 6(2):125-130.

Omprakash, A.V.R, Kumararaf, D.; Narahari, I.A.; Parasad, J. and Sundararasu, V. (1992). Semen characteristics and their correlations with fertility in white Leghorn as influenced by semen diluents. *Indian Vet.J*.69:333-337.

Orakempenichi-pinto, H. Schindle, S. Bornsteu and Maty Boroutchieve (1970). The Fertilization rate of domestic hens after intra magnal or intra-uterine Inseminations with turkey spermatozoa.

Parker, H.M and Mc Daniel, C.D. (2003). Semen Dilution prior to Analysis influences The ability of sperm quality Analyzer to predict fertility whether inseminating with a constant number of sperm or a constant volume of sperm or a constant volume of semen-Poultry Sc.82;1808-1815.

Parker, H.M. and Mc Daniel, C.D. (2005). Immediate Impact of Semen Dilutent and Rate of Dilution on the Sperm quality index, ATP utilization Gas Exchange and ionic balance of breeder sperm, poultry sc. Dept.mississipi 3976.Art.NoJ0683.

Penfold, L. M.; Harnal, V.; Lynch, W.; Bird, D.; Derrickson, S.R. and Widt, D.E. (2001). Characterization of Northern pintail (Anas acuta) ejaculate and the effect of sperm preservation on fertility.

Perry, E. J. (1962). *General information*. In: A. I. of Animals ed. Enos J. Perry. Rutgers Univ. Press. New Brunswick, New Jersey.

Reddy, R.P (1995). *Artificial Insemination of Broilers Economic and Management Implications* .pp 73-89.

Reece, F.N and Lott, B.D. (1993). The effects of temperature and age on body weight and feed efficiency of broiler chickens. *Poult Sc.*, 62:1906-1908.

Renden, J.A.; May, E.B. and Benoff, F.H. (1981). Histochemistry of uterovaginal sperm-host glands in Japanese quail (Coturnix coturnix japonica) with reference to the period of oviposition. *Poultry science* 60,2529-2535.

Rose, S. P. (1997). *Principles of poultry science*. CAB International U.S.A.

Salisbury, G. W., Vandermark, N. L. and Lodge, J. R. (1978). *Physiology of reproduction and artificial insemination in cattle*, 2nd ed. W.H. Freeman and Co., p. 247-417.

Salisbury, G.W. and Mercier, E. (1945). The reliability of estimates of the proportion of morphologically abnormal spermatozoan in bull semen. *J. Animal Sc.*, 4:174-178.

Schuh, H. (2009). Comparison between liquid and deep-frozen semen for artificial Insemination in developing and developed countries.

Scott, T.W.; White, I.G. and Annison, E.F. (1962).Glucose and acetate metabolism by ram, bull, dog and fowl spermatozoa. *Biochemical Journal* 83;398-404.

Sexton, T.I. (1986). Comparison of Commercial diluents for holding turkey semen 24 hours at $4^0$C. *Poult Sci.,* 67:131-134.

Sexton, T.I. (1988). Influence of damaged spermatozoa on the fertility of turkey semen.

Sexton, T.J. (1979). Preservation of poultry semen. A review. pages 159-170 in Beltseville symposia in Agricultural Research, 3. Animal Reproduction.

Shaklee, W.E.; Cruse, C.J.; Chery, N. and Stepen, L. (1980). *Poultry Science*-July 1978 Vol.57, No.4.

Siudzinska, A. and Lwkaszewics, E. (2008). Effect of semen extender and storage time on sperm morphology of four chicken breeds. *J. Appl. Poult. Res*., 12:101-108.

Smith, A.J (1990). *A textbook on poultry production, The tropical Agriculturist Series*. National Academic Press.

Snedecor, G.W. and Cochran (1974). *Statistical method* $6^{th}$ ed. Iowa State University press, Ames Iowa.

SPSS for Windows (2001). Standard version SSPC Inc. London

Sturkie P.D and Mueller, W.I. (1959). *Avian physiology –Reproduction in the female and Egg production*.

Tabataboeis S,R.; Batvani, A. and Talebi, A.R. (2009). Comparison of semen quality of in Indigenous and Ross Broiler Breeded Roosters-*Journal of Animal and Veterinary Advance*, 8(1) 90-93.

Taner, H. and Ergum, A. (2010) Fertilizing ability of short-term preserved spermatozoa. Abant trout (*Salmo trutta abanticus T,*1954) Ankara univ vet.fak.Derg 57,33-38.

Tara Weaver (1998). Spotting top-notch toms-males turkeys. How Researchers are re-Volutionizing turkey production-*Agric.Res*.

Technical Advice Sheet (2006). *Artificial insemination for female turkeys*-British United Turkey Ltd.

Technical Advice sheet (2006). *Semen collection for artificial insemination*.-British United Turkeys.

Thear, K. (2005). Starting with Turkeys, Incubating and Hatching Turkey Eggs_*www.poultry.allotment.org.uk/poultry/start-keepingturkeys/turkey-eggs*.php.Pp 1-3.

Thears, K. (2007). Starting with Turkeys, Breeding Turkeys. Poultry pages. John Hrison. *www.poultry allotment.org.uk/poultry pages.com*

The-Perfect-Turkey .com.2007.Facts Sheet.

Thurston, R.J. (1995) Storage of poultry semen above freezing for 24-48 hours proceedings of the 1st international symposium on the Artificial insemination pp107-122

Thurstons (1995). Storage of poultry semen above freezing for 4-24 hrs.

Ugwu, S.O.C and Igboeli, G. (2009). Motility and fertilizing capacity of boar semen stored in raffia palm (Raffia hookeri) sap extender at $15^0$C-African Journal of Biotechnology, 8(8); 1984-1987. http://www.academic journals.org/AJB

Ugwu, S.O.C. and Oleforu, O. (2009). A practical course on semen collection and processing for Artificial insemination: In manual of Agricultural practical Fegro press Ltd.

Van overbeek, J.; Conklin, M.E.and Blakeslee, A.F. (1941). Factors in Coconut milk essential for growth and development of very young Datura embryos.Sc., 94.350-351.

VanDemark, N.I. (1950). Extension of semen before and after cooling and its effects on fertility. *Anns. Biol. Anim. Biochem. Biophy*. 5:419.

Vegetarian Society (2001). VegSoc UK information sheet. http.//artoflacking. com/IET/HEALTH/TURKEYS.TXT

Waidi, F. S.; Matthew, O. O. and Matthew, O. A. (2007). Coconut milk-Citrate as extender for WAD buck spermatozoa at room temperature. www.bioline.org.br/request/bk0711Vol.19 No.2.

Wheeler, N.J.C. and Andrew, F.N. (1943). The Influence of season production in the domestic fowl. *Poult Sc*. 3.439-473.

Wikipedia (2009). Narragansett-Turkey –http://en.wikipedia.org/wiki/ Narragansett Turkey" Retrieved 2009.

William, K. (2003). The Trauma Filled Lives of Turkey-Agric.Biotechnology-*www.arts.usda.gov/is/ar/archive/jui9*

Wishart, G. (1984). Metabolism of fowl and turkey spermatozoa at low temperatures. *Journal of Reproduction and Fertility* 70,145-149.

Zahradden, D.; Butswat, I.S.R.; Kalla, D.J.U.; Sir, S.M. and Bukar, M.T. (2005). Effect of Frequency of Ejaculation on semen characteristics in Two Breeds of Turkeys (*maleagris galapavo*) Raised in a Tropical Environment. *International Journal of poultry sc*., 4(4):217-221.